DUALITY IN THE ELECTRICAL RESPONSE OF THE LATERAL EYE OF LIMULUS POLYPHEMUS

V. J. WULFF [1]

Marine Biological Laboratory and Department of Physiology, University of Illinois

Photoreceptor activity is accompanied by electrical changes, and the lateral eye of Limulus is no exception. Illumination of this photoreceptor produces a transient change in voltage (Graham, 1932; Hartline, 1928; Hartline and Graham, 1932). This change in voltage, called the retinal action potential, is measurable across the eye, front to back, and its polarity is such that the corneal surface becomes transiently negative to the back of the eye.

The retinal action potential recorded from the lateral eye of Limulus in response to a short period of illumination (Graham, 1932) exhibits a rather simple contour, consisting of a monophasic negative wave which returns to the base line. Close inspection of this response (Fig. 2, Graham, 1932) reveals a small step-like initial elevation which precedes in time the larger negative wave. Heretofore, no study, nor even any mention, has been made of this initial phase of the retinal action potential of the lateral eye of Limulus. The experiments which are the subject of this report describe some of the properties of this initial phase and indicate its possible source.

Materials and Methods

A rectangular piece of the carapace, with the lateral eye in the center, was cut out and dissected free from the underlying tissue. This section of the carapace was mounted in a lucite electrode chamber with a low melting point wax, which also covered all of the outer surface of the carapace, except the cornea. The corneal surface of the eye was bathed by sea water in the forward compartment, in the bottom of which there was a silver-silver chloride electrode. The back of the eye was bathed by sea water in the rear compartment which also contained a silver-silver chloride electrode. The chamber was then covered and placed in the path of a light beam in an otherwise light-tight compartment, and the silver-silver chloride electrodes were connected to the grids of the amplifier. One hour in the dark was found to be sufficient for complete dark-adaptation.

The light source was a 500 watt tungsten filament projection bulb. The light beam passed through 8 cm. of water and a heat absorbing filter. The intensity of the light beam was controlled by Wratten neutral tint filters. The maximum intensity incident on the cornea was 27,000 foot candles, which was considered as unit intensity. The duration (0.01 sec.) of the light flash was controlled by a photographic shutter. A portion of the light beam activated a photocell, the output of which was recorded as the stimulus signal. The potentials were fed into high-gain, condenser-coupled amplifiers and recorded on an ink writer. The frequency

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response of the ink-writer is flat to 100 cps. The amplifiers were operated at different time constants, in order to accentuate the duality of the response. The nominal time constants were calibrated with a square wave voltage pulse.

After dark adaptation of the excised eye, a flash of light of the lowest intensity was admitted and the response recorded. Ten minutes later a second flash of light was admitted and so on until the desired intensity range was covered. When a second series was desirable, an hour was again permitted for completion of dark adaptation and the sequence was repeated.

The effect of light-adaptation upon the electrical response of the excised eye was determined by admitting a test flash of light 5 seconds after the end of a controlled period of exposure to light. The intensity of and exposure to the adapting light could be readily varied.

A third experimental procedure consisted of the introduction of substances into the sea water bathing the eye. These materials were introduced under dim ruby-red light. Recovery from the exposure to red light was permitted from 2 to 7

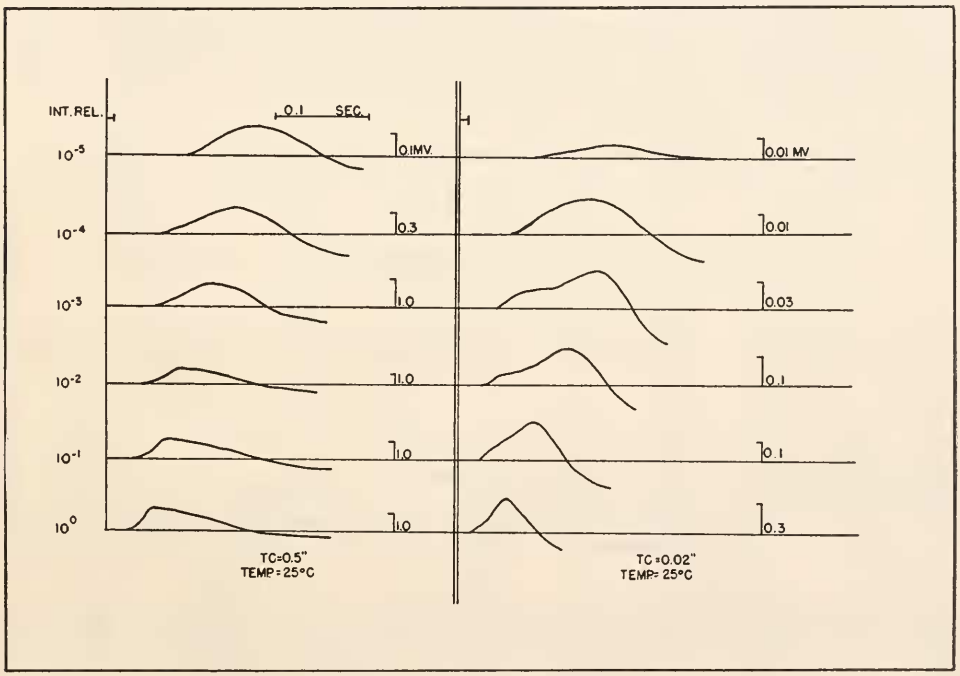

FIGURE 1. Protocols of typical experiments with the lateral eye of Limulus. The original records were reenforced with black ink for photographic reproduction.

Left. A series of responses elicited by test flashes of 0.01″ duration (indicated in the upper line only) and of intensities (10° represents 27,000 foot candles) indicated in the left hand column (int. rel.) opposite the appropriate records. These responses were recorded with 0.5″ time constant. Upward deflection indicates negativity of the corneal electrode. Calibration signal (millivolts) follows each record. The time base is indicated above the upper record.

Right. A similar series of responses recorded with a short time constant (0.02″) to emphasize the dual nature of the response. Exposure, intensity, calibration and time base as above.

minutes, depending upon the intensity of the next test flash. In control experiments, the exposure to red light did not affect the magnitude or contour of the response to a test flash administered immediately after the exposure to the red light, presumably because the intensity of the test flash was sufficiently high.

Forty-eight experiments were conducted at room temperature, which varied from experiment to experiment in the range 25° to 28° C. The crystograph pens recorded with red ink, which had to be reenforced with black india ink for photographic reproduction.

Results

1. Results obtained with 0.5″ time constant

The responses recorded from an excised Limulus lateral eye, stimulated with light flashes of different intensities, exhibit a rather uniform contour and a progressive increase in magnitude (a typical series is illustrated in Fig. 1, left). The relation of retinal action potential magnitude (measured from base line to peak of the negative wave) to the common logarithm of the stimulating intensity (Fig. 2) indicates a tendency to level-off at low and high intensities, with a rather linear intermediate portion. The latency of the response decreases with increasing intensity (Fig. 2, crosses).

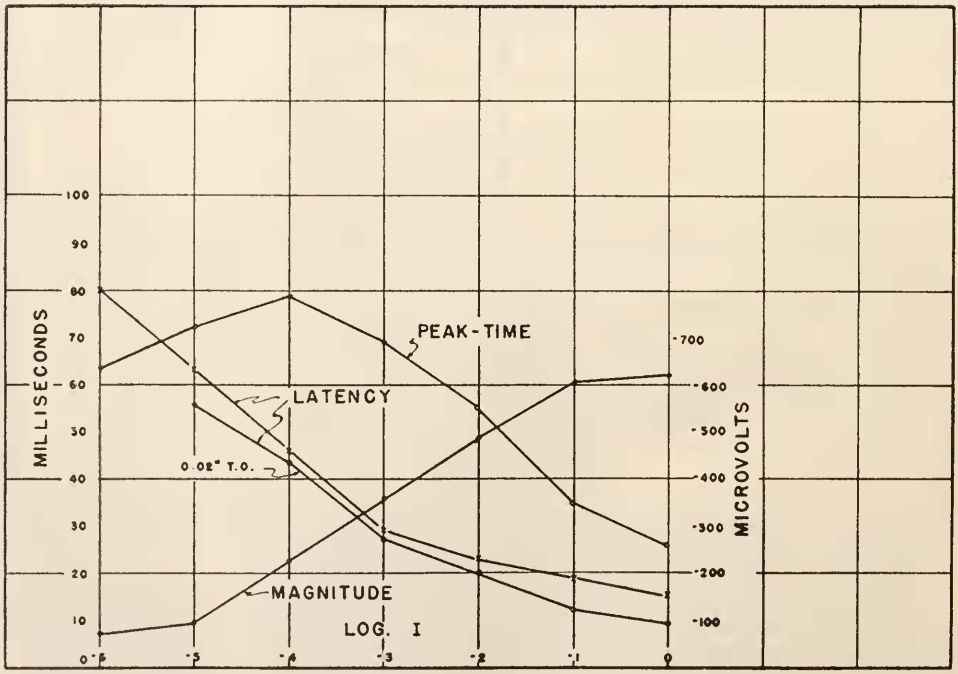

Figure 2. Graphs relating latency, peak-time and magnitude of the retinal electric response to the logarithm (base 10) of the stimulating light intensity. Log 0 represents an intensity of 27,000 foot candles. All data were obtained from responses recorded with 0.5″ time constant, except latency curve marked 0.02″ T. C.

2. Results obtained with 0.02" time constant

These responses were obtained in a manner similar to the above and a typical series from one experiment is illustrated in Figure 1, right. The presence of two phases in the electrical response to flash intensities of 1×10^{-3} and greater is apparent. From such records and others not shown, in which the resolution of the two phases was even greater (Fig. 4, left, lower line), it was possible to measure the magnitudes of the two phases. The relation of these magnitudes to the common logarithm of the flash intensity is shown in Figure 3 (1, magnitude of the first phase; 2, magnitude of the second phase). The departure of this magnitude contour from the more conventional one of Figure 2 may be attributed to the short time constant, as discussed below.

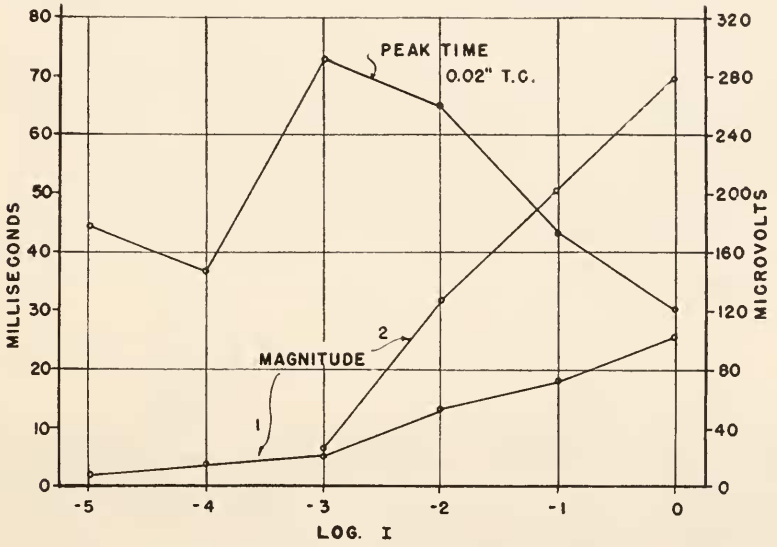

FIGURE 3. Graphs relating peak-time and magnitude of the retinal electric response to the common logarithm of the stimulating light intensity. These data are from responses recorded with a short (0.02") time constant. The magnitude relation marked 1 pertains to the initial phase; that marked 2 pertains to the second phase of the retinal response.

The responses to flash intensities of 1×10^{-4} and 1×10^{-5} (Fig. 1, right) are of uniform contour and do not indicate two phases. The gradual regression, in time, of the second phase (Fig. 1, right) from the lower record on up, suggests another measurement; namely, the time required for the response to reach maximum, hereafter called peak-time. Peak-time is measured from the beginning of the response to its maximum. The relation of peak-time to the common logarithm of the intensity is illustrated in Figure 3 (short time constant) and in Figure 2 (longer time constant).

The latency of the initial phase of the retinal action potential decreases progressively with increasing intensity, giving the relation shown in Figure 2 (0.02" T. C.).

3. Effect of light adaptation

The effect of light adaptation on the retinal electric response, using the short time constant, is illustrated in Figure 4, left. Within the particular conditions of this experiment, it is apparent that light adaptation of 200 foot-candle seconds renders the initial phase invisible and causes some reduction of the magnitude of the second phase. These effects are quite reversible, as indicated in the control record, obtained 10 minutes after the preceding rsponse.

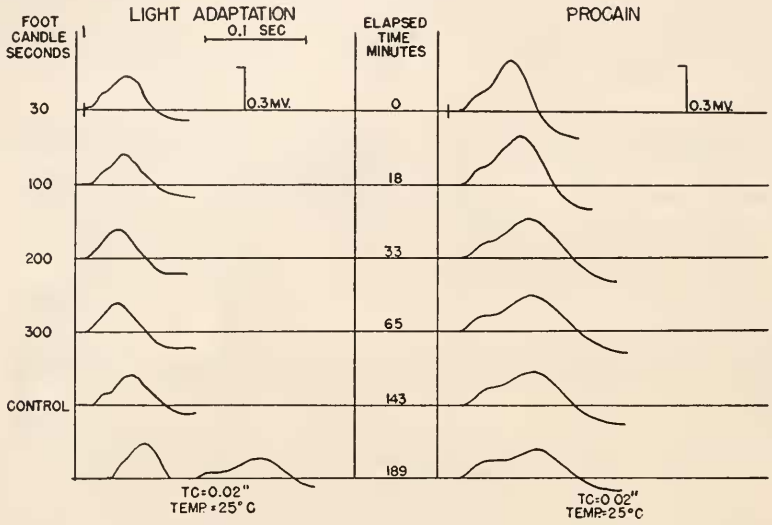

FIGURE 4. Protocols of experiments illustrating the effect of light adaptation (left) and procain (right) on the retinal electric response of the Limulus lateral eye elicited by a constant intensity, constant duration test flash (270 f. c., 0.01″). Column marked "foot candle seconds" indicates the exposure to which the eye was subjected preceding the test flash, which was administered 5 seconds after the adapting light was cut off. Column marked "elapsed time minutes" indicates time elapsed (eye in dark) after the drug was administered. Lower line of left column illustrates the extremes of dissociation of the two phases encountered during these experiments. Amplification and paper speed were constant throughout and are indicated on upper line.

4. The effect of procain hydrochloride

The addition of procain hydrochloride to the sea water in the rear compartment of the eye chamber, in amounts to bring the final concentration to 0.02 per cent, produced immediate cessation of optic nerve activity and effected a gradual reduction in the magnitude of the second phase of the retinal response (Fig. 4, right). The magnitude of the initial phase is but little affected, even in responses (not shown) recorded six hours after introduction of the drug.

5. Variability of dissociation of the two phases

The extent to which the two phases of the retinal action potential are summated varies somewhat from preparation to preparation (Fig. 4, left column, lowest line).

The dissociation of the phases in any one preparation was rather constant throughout. The presence or absence of the rudimentary eye (the mass of white pigment behind the lateral eye) did not affect the two phases of the retinal action potential in any way.

Discussion and Conclusions

1. Is the double response an artifact?

a) Excised lateral eye preparations kept for 8 hours at room temperature generally showed the onset of deterioration by a gradual reduction in magnitude of response. Both phases of the retinal action potential were affected. Preparations kept at room temperature for 20 hours often were totally unresponsive. Apparently both the initial and the second phase are characteristic of living eyes.

b) The dual response has been recorded with silver-silver chloride electrodes and with salt bridges leading to calomel half-cells. The dual response is also apparent in published records (Fig. 2 of Graham, 1932) obtained with an entirely different amplifying and recording system. The duality of the electrical response cannot be considered an artifact attributable to the recording electrode system.

c) The initial phase disappears upon light adaptation (Fig. 4, left) and reappears upon dark adaptation. The initial phase appears in time after a characteristic latency which varies with the intensity of the incident illumination (Fig. 2). These facts indicate that the duality of the retinal action potential is characteristic of the functional photoreceptor.

2. Which phase persists at low intensities?

Measurements of the time from the onset to the crest of the retinal action potential (peak-time) plotted as a function of the log intensity (Fig. 3) reveal a marked discontinuity between log $I = -3$ and log $I = -4$. This indicates that the crest of the response to a flash intensity of 10^{-4} occurs earlier in time than the crest of the response to a flash intensity of 10^{-3}, which is apparent also in Figure 1, right column. Scanning of these records from bottom to top indicates that the crests of the responses to flash intensities of 10^{0} to 10^{-3} are the crests of the second phase. At 10^{-4} only one phase is present and, since the crest occurs earlier in time, it is implied that this is the crest of the initial phase. The second phase is apparently absent at this and lower intensities.

A similar conclusion follows from consideration of the magnitude-log intensity relationships of Figure 3. The magnitude of the second phase (2) decreases rapidly from 10^{0} to 10^{-3}. Extrapolation of this curve would indicate a complete absence of the second phase in response to flash intensities of 10^{-4} or lower. It must be suggested, however, that recording with a short time constant sacrifices a large part of the transient voltage change, particularly that associated with the second phase. This undoubtedly produces some distortion of the magnitude-log I relation (compare with a similar curve, Fig. 2). The initial phase, on the other hand, shows a gradual increase in magnitude and the maximum at log $I = 0$ is appreciably less than the magnitude of the second phase. Such data were obtained from six experiments where the degree of dissociation was sufficient to make these measurements possible throughout the entire intensity range.

3. The significance of the two phases

The rapid disappearance of the initial phase upon light adaptation, coupled with the fact that low intensity light flashes elicit only the initial phase, suggests that this response may be associated with receptor cells of greater sensitivity than those which give rise to the second phase. This consideration implies that the lateral eye of Limulus contains two functionally different kinds of sense cells. It is of great interest that two structurally different kinds of sense cells have been described for the lateral eye of Limulus (Demoll, 1914). The complex eye of Limulus is com-

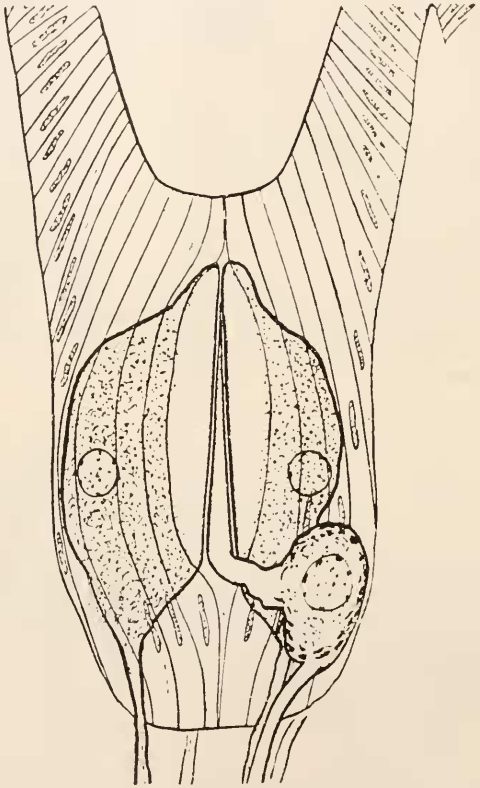

FIGURE 5. Diagrammatic representation of a longitudinal section through an ommatidium from the lateral eye of Limulus showing the radial retinal cells and the eccentric retinal cell with its central process, all embedded in an epithelial framework. (From Demoll, 1914.)

prised of many visual units, the ommatidia. All ommatidia, according to Demoll (1914), possess a similar spatial arrangement of receptor cells (Fig. 5). There are a group (10–15) of receptor cells radially arranged about the longitudinal axis of the ommatidium, leaving a hollow core in the center. This core is partially occluded by a process originating from an eccentrically located sense cell (Fig. 5). In each ommatidium there is only one eccentric sense cell and this seldom contains pigment, whereas the radially located sense cells often contain pigment (Demoll,

1914). The relative paucity of the eccentric sense cells, their centrally located processes and their freedom from pigment makes them the likely source of the initial phase of the retinal action potential. Further, in certain experiments the magnitude of the second phase of the retinal action potential has exceeded 1 millivolt, whereas the initial phase rarely exceeded 0.1 millivolt. This response magnitude ratio of 10 to 1 roughly parallels the population ratio of radially arranged sense cells to eccentric sense cells, 10—15 to 1. It is concluded, therefore, that the response of the eccentric sense cells to illumination produces the initial phase and the response of the radial sense cells produces the second phase of the retinal action potential.

The presence of two morphologically and physiologically different types of receptor elements in the lateral eye of Limulus is reminiscent of the dual nature of most vertebrate retinae.

Summary

Retinal action potentials recorded from the excised lateral eyes of Limulus, using a short time constant amplifier coupling, show a dissociation into two discrete responses, an initial and a secondary phase, over part of the intensity range. Analysis of the data indicates that the single response present at low intensities of stimulation is the initial phase and that both phases are present only in response to higher intensities of illumination. This sequence of single and dual phases with increasing intensity of stimulation gives rise to the peculiar peak-time relation shown in Figure 3. Light adaptation causes a rapid disappearance of the initial phase and only a gradual disappearance of the second phase. The magnitude of the initial phase is much smaller than the magnitude of the second phase. These data suggest that the initial phase represents the response of highly sensitive receptors (eccentric retinula cells) that are outnumbered by less sensitive receptors which contribute to the second phase (the radial retinula cells) (Demoll, 1914). Procain hydrochloride administered to the excised eye results in a reduction in magnitude of phase 2, and little or no change in magnitude of phase 1.

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